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Characterization of integrative and conjugative elements carrying *erm*(B) and *tet*(O) resistance determinants in *streptococcus uberis* isolates from bovine milk in Chiba prefecture, Japan: CompArative GEne cluster analysis toolbox with ICEfinder

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Abstract

Objective We aimed to characterize integrative and conjugative elements (ICEs) in antimicrobial resistant *Streptococcs uberis* isolates from bovine milk in Chiba, Japan, based on whole-genome sequence (WGS) data.

Results Of the 101 isolates, we found the 36 isolates harboring *erm*(B)–*tet*(O), showing resistance to macrolides–lincosamides–tetracyclines. The 22 isolates were randomly selected and subject to WGS determination. The genomes measured 1.991–2.517 Mbp, with G + C contents of 35.8–36.9%. We used ResFinder–ICEfinder (web-based applications) to search for the antimicrobial resistant genes and ICEs. ResFinder detected combined *erm*(B)–*tet*(O)–*ant(6)-Ia* at the identical contig in each WGS. ICEfinder detected ICEs belonging to the same contigs, which contained *erm*(B)–*tet*(O)–*ant(6)-Ia* complete or partial sequences. Detection of putative ICEs using comparative genomic analysis was performed with identification of other streptococcal ICE resembling *S. uberis* ICEs. Using comparative genomic analysis (a reference WGS in NZ01 strain), putative ICE base size in UB37 isolate was 77,386-bp that was identical in other 13 isolates. Another similar streptococcal ICE was *S. suis* ICE*nsui78*–*tet*(O)–*erm*(B) mobile element. For ICE characterization in *S. uberis* with WGSs, a comparative genomic analysis is required with use of ICEfinder and other annotation tools.

Keywords Integrative and conjugative element, *Erm*(B), *Tet*(O), *Streptococcus uberis*, CompArative GEne cluster analysis Toolbox, ICEfinder

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Introduction

Mobile genetic elements (MGEs) play a major role in horizontal gene transfer among microorganisms. Integrative and conjugative elements (ICEs) of MGEs are self-transmissible in bacteria and are characterized by both integrative and conjugative features [1]. ICEs are mosaic elements with both bacteriophage- and plasmid-like characteristics that integrate into and replicate with host cell chromosomes [2]. Additionally, ICEs transfer their antimicrobial resistance (AMR) determinants as well as the genes involved in their mobility/regulation/maintenance. Many ICEs are demonstrated among the genus *Streptococcus* [3]. ICEs belonging to Tn*916*-Tn*1545* family transfer the widely disseminated tetracycline-resistance determinant *tet*(M), along with additional determinant *erm*(B) [4]. *erm*(B) gene encodes methylase that generates a posttranscriptional methylation of 23 S rRNA, leading to cross-resistance to macrolides, lincosamides, and streptogramin B antimicrobials [5].

Bovine clinical/subclinical mastitis in dairy cattle affect udder health, milk quality, and milk production, resulting in economic losses in dairy farms. Major causative microorganisms of the mastitis contain *Escherichia coli*, *Staphylococcus aureus* [6], coagulase-negative staphylococci, and contagious/environmental streptococci [7]. Contagious *Streptococcus* is *S. agalactiae*, whereas environmental streptococci contain *S. dysgalactiae*, *S. canis*, and *S. uberis* [8, 9].

S. uberis is isolated from environmental sources (soil, pasture, bedding materials, and bovine feces) and is present on the skin surface of dairy cows [8]. This microorganism develops bovine (sub)clinical mastitis during lactating/non-lactating periods after direct contact with the teat apex [10]. Molecular epidemiological investigation regarding *S. uberis*-associated clinical mastitis in dairy herds described that either predominant or a limited number of isolates may produce intramammary infections or transmission among cows (including potential transmission of the isolates via milking machine/environment) [11].

Macrolides, lincosamides, and beta-lactams are administered for treatment of *S. uberis*-associated mastitis [12]. Excessive usage of antimicrobials in dairy herds can result in elevated AMR among mastitis bacteria [13]. Zhang et al. [12] reported AMR phenotypes/genotypes among *S. uberis* isolates from bovine mastitis in Thailand. The isolates were resistant to tetracycline (82.0%), ceftiofur (19.3%), and erythromycin (8.3%). Prevalent AMR genes were *tet*(M) (87.3%), *erm*(B) (66.2%), and *blaZ* (6.6%). We have showed AMR patterns of *S. uberis* from bovine milk in Japan [14]. AMR monitoring of bovine mastitis-associated *S. uberis* may support an antimicrobial stewardship program for dairy farms.

Based on the findings by polymerase chain reaction (PCR) method, Haenni et al. [15] documented diverse and mobile features of ICEs among bovine *S. uberis* isolates. We searched for related articles by entering the keywords "streptococcus uberis, integrative and conjugative element, whole-genome" in PubMed database (https://pubmed.ncbi.nlm.nih.gov/) [16]. There is limited literature available on this topic, although sequence characterization and novel insights into bovine mastitis-associated *S. uberis* in dairy herds have been reported [17]. The aim of our work is to characterize ICE characterization in *S. uberis* AMR isolates from bovine milk based on whole-genome sequence (WGS) data.

Methods

AMR phenotyping/genotyping

All bovine milk-origin isolates ($n=106$) identified based on mass spectrometry results were provided by Sanritsu Zelkova Veterinary Laboratory during Mar–Oct 2022. This laboratory determined minimum inhibitory concentrations of 14 antimicrobials (penicillin-G/ampicillin/minocycline/erythromycin/azithromycin/clindamycin/levofloxacin/chloramphenicol/cefotaxime/ceftriaxone/cefepime/cefozopran/meropenem/vancomycin) using broth microdilution method (MICroFAST Panel Types 7 J for *Streptococcus* spp.; Beckman Coulter Inc., Tokyo, Japan) recommended in Clinical and Laboratory Standards Institute (CLSI) M100-S26 document for alpha-hemolytic streptococci [18]. Quality control was performed using *Enterococcus faecalis* American Type Culture Collection (ATCC) 29,212, *Streptococcus pneumoniae* ATCC 49,619, and *S. uberis* ATCC 700,407 strains with the antimicrobial susceptibilities. To determine susceptibility/resistance to minocycline, we used tetracycline breakpoints in accordance with the CLSI guidelines.

One isolate per host was stored at −70 °C to −80 °C in Kitasato laboratory. We performed PCR methods to differentiate *S. uberis* and *S. parauberis* according to PCR fragments of 16 S and 23 S rRNA genes [19]. One hundred one isolates were analyzed. AMR genotyping [*blaZ*–*erm*(A)–*erm*(B)–*mef*(A)–*lnu(*B*)*–*lnu(*D*)*–*tet*(M)–*tet*(O)–*tet*(K)–*tet*(L)–*tet*(S)] was conducted using PCR assay [20, 21].

Randomized selection of *S. uberis* isolates with *erm*(B)–*tet*(O)

We found the isolates harboring both *erm*(B) and *tet*(O) ($n=36$): all isolates indicated resistance to macrolides, lincosamides, and tetracyclines (the AMR rates of 33.7%, 54.5%, and 48.5%) [14]. Combined *erm*(B)–*tet*(O) genotype seems to be a representative marker to find ICEs in corresponding isolates, because both genes are located within MGEs [15]. The twenty-two isolates with

erm(B)–*tet*(O) were selected based on random sampling numbers using Excel application. *S. uberis* ATCC 700,407 having WGS (https://genomes.atcc.org/genomes/26250c65fb774abc) at ATCC site was used as a reference strain. The enrolled strains' list is shown in Supplementary Table 1.

WGS determination

DNAs from the twenty-two isolates were extracted as previously reported [22]. Whole-genome sequencing was carried out on a DNBSEQ-G400RS platform (MGI-Tech, Tokyo, Japan) using DNA Nanoball technology [23]. Sequencing library was generated using MGIEasy FS DNA Library Prep Set. Paired-end runs were performed with a read length of 2×150-bp.

We assessed read lengths, ambiguous bases, and quality scores for quality control. The reads were trimmed using quality trimming tool in CLC Genomics Workbench (v.8.0.2) with default parameters (quality limit and maximum number of ambiguous bases). *De novo* assembly was conducted using CLC Genomics Workbench with modified parameters, wherein the minimum contig length was set to 800-bp. Draft genome sequences were annotated using DDBJ Fast Annotation and Submission Tool (DFAST; https://dfast.nig.ac.jp) [24]. We determined assembly metrics and annotated features including genome size, number of contigs, average coverage, N_{50}, numbers of coding DNA sequences (CDSs)/tRNAs/rRNAs/clustered regularly interspaced short palindromic repeats (CRISPR), G+C content, and coding ratio.

Additionally, the sequence type/clonal complex were determined using *S. uberis* PubMLST Isolate database (https://pubmlst.org/bigsdb?db=pubmlst_suberis_isolates). We also constructed the phylogenetic tree of 22 isolates and ATCC 700,407 as previously reported [25].

Web-based applications

We used ResFinder v.4.1 (https://cge.food.dtu.dk/services/ResFinder/) and ICEfinder v.1.0 (https://bioinfo-mml.sjtu.edu.cn/ICEfinder/index.php) with WGSs, to comprehensively search for related AMR genes and ICEs [25, 26]. Among the ICEs found, we selected the ICE containing *erm*(B)–*tet*(O) genes as a candidate from each isolate for further characterization.

Detection of putative ICEs using comparative genomic analysis

Flanking products of each candidate ICE were confirmed based on DFAST annotations. We selected a complete *S. uberis* WGS sequence without combined *erm*(B)–*tet*(O) as a reference. This WGS needed to possess flanking products identical to those of selected ICE based on the ICEfinder results. When we subtracted flanking products in reference WGS from corresponding products in candidate WGSs (comparative genomic analysis), we could detect putative ICEs containing *erm*(B)–*tet*(O). The subtraction procedure was based on concept "integration" resulting from ICE definition.

The putative ICE base size and product organization were determined for each WGS. To conduct homologous gene cluster searches and to generate publication-quality figures of gene cluster comparison between reference and candidate WGSs, we applied CompArative GEne Cluster Analysis Toolbox (CAGECAT, https://cagecat.bioinformatics.nl) consisting of two components of cblaster (for searches) and clinker (for figures) [27–29]. A Prokaryotic Genomes Annotation Pipeline (PGAP) in National Center for Biotechnology Information (NCBI) was applied for re-annotation. To specify ICE components, ICEscreen software was also applied [30]. To speculate protein function similarities, we used basic local alignment search tool x (blastx, translated nucleotide to protein homology search) on NCBI.

We found out where ICE*UB37* was inserted into the genome selected.

Searching for other streptococcal ICE resembling *S. uberis* ICEs

Product organization of each determined ICE was inserted into homology search box on NCBI site. Other streptococcal ICEs with high-percent identity with the inserted query were extracted. One ICE showing the high-percent identity with inserted ICEs in our 22 isolates was specified. Using clinker, we constructed a visualized and interactive figure among the specified ICE and our ICEs.

Results

Assembly metrics and annotated features

WGS assembly metrics and annotated features (genome size, number of contigs, average coverage, N_{50}, numbers of CDSs/tRNAs/rRNAs/CRISPR, G+C content, and coding ratio) are shown in Supplementary Table 2.

Additionally, the sequence type/clonal complex are shown in Supplementary Table 2. Of 22 strains, twenty belonged to CC996, suggesting that this population is closely related. The constructed phylogenetic tree of 22 isolates and ATCC 700,407 is shown in Supplementary Fig. 1.

Web-based application results

Web-based application results with WGSs (AMR genes and ICEs) are shown in Supplementary Table 3. There were combined genes *erm*(B)–*tet*(O)–*ant(6)-Ia* located at identical contig for each WGS. We observed other AMR genes [*lnu*(C), *lsa*(E)–*lnu*(B), or *lnu*(C)–*fosB*] at different contigs of WGSs in three isolates (UB25, UB78, or UB97). ICEfinder detected ICEs belonging to the same contigs

containing combined *erm*(B)–*tet*(O)–*ant(6)-Ia* complete or partial sequences. All ICEs included *erm*(B)–*ant(6)-Ia* complete sequences, whereas some possessed *tet*(O) complete sequence: others had *tet*(O) partial sequence (Supplementary Table 3). Sometimes ICEfinder does not delimit the ICEs correctly and misses some part of the ICE.

Detection of putative ICEs using comparative genomic analysis

Detection approach of putative ICEs using comparative genomic analysis is shown in Fig. 1. As a reference WGS, we selected a complete *S. uberis* sequence (nucleotide accession no. CP022435.1) in bovine mastitis-associated NZ01 strain from New Zealand in 2014. UB37 isolate was chosen as an analyzed WGS. NZ01 and UB37 WGSs shared identical flanking products of 8-oxo-dGTP diphosphatase and RlmD [23 S rRNA (uracil(1939)-C(5))-methyltransferase].

Using comparative genomic analysis, the putative ICE base size of UB37 WGS was considered 77,386-bp, and the product organization of ICE*UB37* was determined (Fig. 1). This ICE contained site-specific DNA-methyltransferase, serine integrase, relaxase MOBP (PF03432), MobC family plasmid mobilization relaxosome protein, SAG1252 family conjugative relaxosome accessory protein, SNF2-related protein, phage tail tip lysozyme, conjugal transfer protein TrbL, DNA (cytosine-5-)-methyltransferase, and replication initiator protein A. Using ICEscreen, we confirmed that ICE*UB37* included a component set consisting of VirB4 and coupling protein VirD4. Its *virB4* and *virD4* sequences (sizes 2,352-bp and 1,818-bp) were similar with those of *S. suis* D9 complete genome (nucleotide accession no. CP002641.1) belonging to the Tn*5252* (Supplementary Fig. 3). Furthermore, using blastx, we found that conjugal transfer protein TrbL has VirB6 function and that phage tail tip lysozyme has in part peptidoglycan hydrolase PcsB in *Streptococcus*.

Supplementary Fig. 2 indicates where ICE*UB37* was inserted into the NZ01 genome.

Similarities/differences in ICE results (base start to end and size) between comparative genomic analysis and ICEfinder are shown in Table 1. There were two prevalent ICE base sizes by comparative genomic analysis

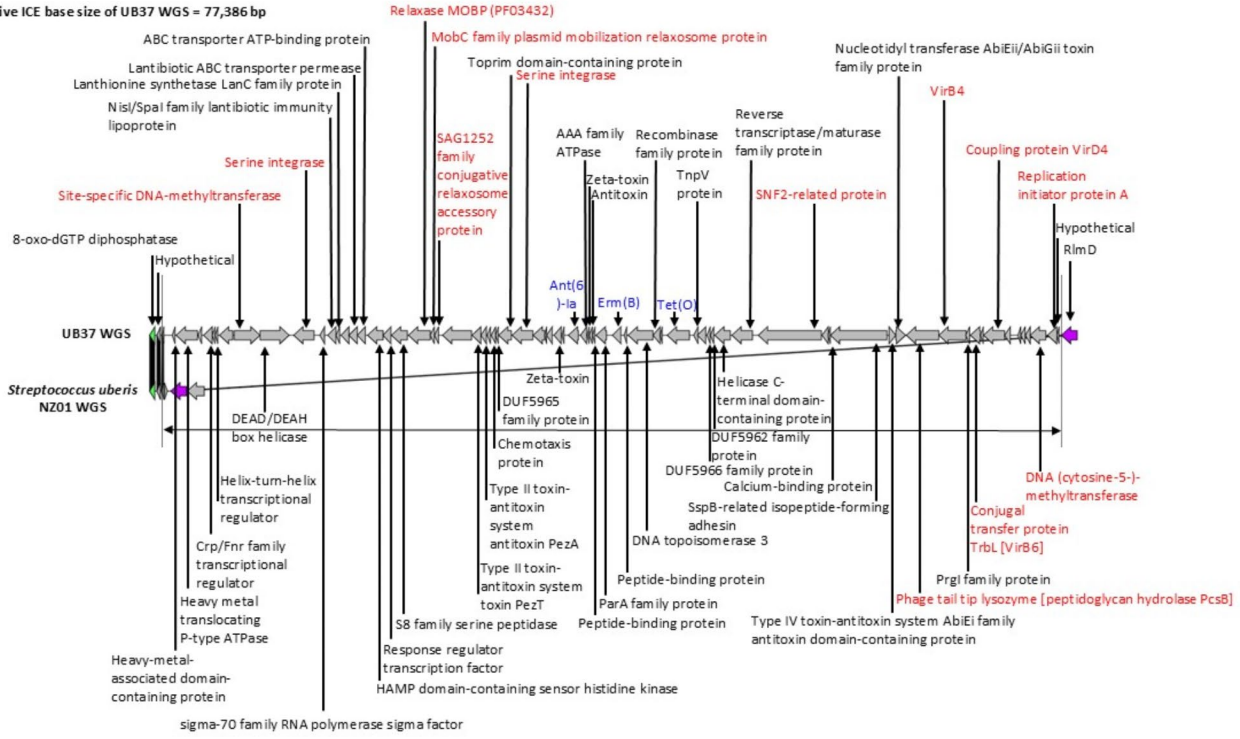

Fig. 1 Detection approach of putative Integrative and conjugative elements (ICEs) using comparative genomic analysis. As a reference, we applied a complete genome sequence (nucleotide accession no. CP022435.1) in bovine NZ01 strain from New Zealand in 2014: UB37 isolate was chosen. Similar/identical product comparisons between reference and candidate WGSs are shown in the same colors. Black gradations in interspace among the corresponding genes indicate percent identity. NZ01 and UB37 WGSs possessed identical flanking products of 8-oxo-dGTP diphosphatase and RlmD [23 S rRNA (uracil(1939)-C(5))-methyltransferase]. Putative ICE base size was 77,386-bp. Product organization in ICE was determined. The core products are shown in red letters; the antimicrobial resistance products are shown in blue letters. We performed the re-annotation for ICE*UB37* using a Prokaryotic Genomes Annotation Pipeline. To specify ICE components, ICEscreen was applied. To speculate protein function similarities, we used basic local alignment search tool x; the protein functions are shown in brackets

Table 1 Similarities/differences in ICE results between ICEfinder and comparative genomic analysis

Isolate	ICE base start to end [contig no.] by ICEfinder	ICE base start to end [contig no.] by CAGECAT	ICE base size by ICEfinder (bp)	ICE base size by CAGECAT (bp)
UB2	244,514 to 294,228 [1]	224,660 to 302,024 [1]	49,715	77,365
UB7	244,883 to 294,618 [5]	225,029 to 302,414 [5]	49,736	**77,386**
UB15	854,622 to 902,726 [3]	858,346 to 935,731 [3]	48,105	**77,386**
UB23	577,061 to 640,616 [3]	572,095 to 643,092 [3]	63,556	70,998
UB25	820,660 to 868,764 [1]	824,384 to 901,769 [1]	48,105	**77,386**
UB36	244,515 to 294,250 [1]	224,661 to 302,046 [1]	49,736	**77,386**
UB37	818,668 to 866,772 [3]	822,392 to 899,777 [3]	48,105	**77,386**
UB38	818,651 to 866,755 [5]	822,375 to 899,766 [5]	48,105	77,392
UB41	244,729 to 294,464 [3]	224,875 to 302,260 [3]	49,736	**77,386**
UB56	819,593 to 867,697 [2]	823,317 to 900,702 [2]	48,105	**77,386**
UB58	244,515 to 294,250 [3]	224,661 to 302,046 [3]	49,736	**77,386**
UB63	781,903 to 830,007 [3]	785,627 to 863,012 [3]	48,105	**77,386**
UB68	789,597 to 874,926 [2]	818,820 to 895,554 [2]	85,330	76,735
UB77	576,689 to 657,436 [1]	582,527 to 659,912 [1]	80,748	**77,386**
UB78	771,598 to 857,048 [1]	800,942 to 878,348 [1]	85,451	77,407
UB81	244,514 to 294,249 [1]	224,660 to 302,045 [1]	49,736	**77,386**
UB82	819,632 to 867,736 [1]	823,356 to 900,741 [1]	48,105	**77,386**
UB85	789,395 to 874,724 [1]	818,618 to 895,352 [1]	85,330	76,735
UB89	815,907 to 864,011 [2]	819,631 to 897,016 [2]	48,105	**77,386**
UB92	188,482 to 268,578 [2]	194,320 to 271,054 [2]	80,097	76,735
UB97	819,497 to 867,601 [3]	823,221 to 900,606 [3]	48,105	**77,386**
UB99	817,430 to 870,565 [2]	823,268 to 872,342 [2]*	53,136	> 49,075

*Accurate estimation of UB99 ICE was impossible because of the analyzed position at contig terminal

Most prevalent ICE base size by comparative genomic analysis is shown in bold letters

consisting of 77,386-bp (UB7/UB15/UB25/UB36/UB37/UB41/UB56/UB58/UB63/UB77/UB81/UB82/UB89/UB97) and 76,735-bp (UB68/UB85/UB92). In contrast, we found three prevalent ICE base sizes by ICEfinder containing 48,105-bp (UB15/UB25/UB37/UB38/UB56/UB63/UB82/UB89/UB97), 49,736-bp (UB7/UB36/UB41/UB58/UB81), and 85,330-bp (UB68/UB85).

However, we obtained partial ICE base size (> 49,075-bp) in UB99. The accurate estimation was impossible because of the analyzed position at contig terminal (Table 1/Fig. 2) (see the **Limitations**).

Putative identification of other streptococcal ICE resembling *S. uberis* ICEs

Putative identification of other streptococcal ICE resembling *S. uberis* ICEs (UB37/UB68/UB23/UB99) is shown in Fig. 2. Another similar streptococcal ICE was *Streptococcus suis* strain STC78 ICE*nsui78*–*tet*(O)–*erm*(B) mobile element (nucleotide accession no. ON944185.1; 55,758-bp), based on the similar product organization between UB37/UB68/UB23/UB99 ICEs and ICE*nsui78*. In contrast, this element did not possess arrangement of Ant(6)-Ia.

Discussion

Firstly, ICEfinder was applied to comprehensively search for ICEs. Some ICEs possessed *tet*(O) complete sequence, whereas others had *tet*(O) partial sequence (Supplementary Table 3), although phenotypic resistance to minocycline was confirmed in all isolates (Supplementary Table 1). This suggests that ICEfinder did not consider the entire ICE. Secondary, we performed putative ICE detection using comparative genomic analysis (a reference WGS in NZ01 strain). Twenty-one ICEs were determined (Table 1/Fig. 2). We could not obtain the accurate results of UB99 ICE because of the analyzed position at contig terminal. Therefore, we should construct complete WGSs using short- and long-read sequencing as we previously reported complete WGSs of *Streptococcus canis* strains from dogs [31].

The facts that ICE*UB37* can be found in other streptococci is significant topics for discussion. Huang et al. [2] reported genomic organization of ICE*Sa2603* (nucleotide accession no. AE009948.1; 54,349-bp) in *S. agalactiae* strain 2603 V/R. ICE*Sa2603* comprised three proteins of SAG1250 (similar with prokaryotic DNA relaxase), SAG1251 (similar with MobC of prokaryotic plasmid), and SAG1252: ICE*UB37* contained SAG1250 family conjugative relaxase [relaxase MOBP (PF03432)], MobC

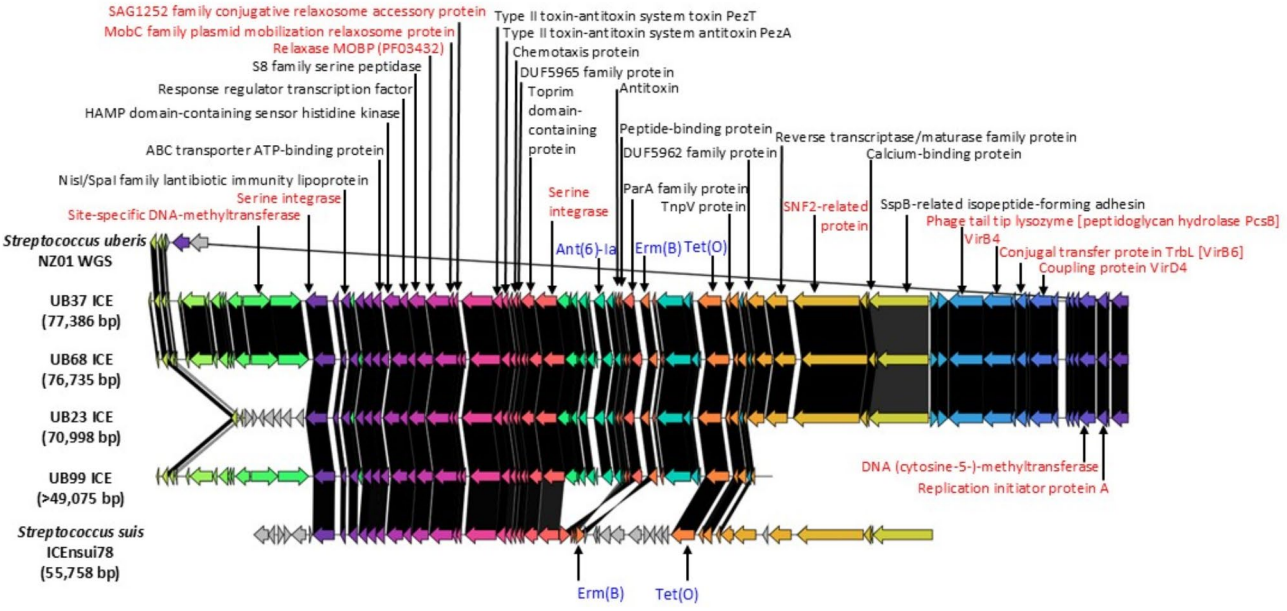

Fig. 2 Putative identification of other streptococcal Integrative and conjugative element (ICE) resembling *S. uberis* ICEs (UB37/UB68/UB23/UB99), with *S. uberis* NZ01 WGS. Similar/identical product comparisons between reference and candidate WGSs are shown in the same colors. Black gradations in interspace among the corresponding genes indicate percent identity. Another similar streptococcal ICE was *Streptococcus suis* strain STC78 ICE*nsui78*–*tet*(O)–*erm*(B) mobile element (nucleotide accession no. ON944185.1), based on the similar product arrangements between UB37/UB68/UB23/UB99 ICEs and ICE*nsui78*. The core products are shown in red letters; the antimicrobial resistance products are shown in blue letters. We performed the re-annotation for ICE*UB37* using a Prokaryotic Genomes Annotation Pipeline. To specify ICE components, ICEscreen was applied. To speculate protein function similarities, we used basic local alignment search tool x; the protein functions are shown in brackets

family plasmid mobilization relaxosome protein, and SAG1252 family conjugative relaxosome accessory protein. We found the similarity in ICE component involved with mobilization between ICE*Sa2603* and ICE*UB37*. A structure, which was very similar with ICE*UB37* [66,036/66,071 (99.95%)], was also found in *S. ruminantium* WGS (nucleotide accession no. AP025333.1; 67,641-bp spanning nucleotides from 1,168,946 to 1,236,586), using homology search in NCBI nucleotide database. *S. ruminantium* is the causative agent of several bovine and ovine diseases and *S. ruminantium*-related sheep mastitis outbreak has recently been reported from Italy [32]. Another structure similar with ICE*UB37* [33,405/34,281 (97.44%)] was observed in *S. pluranimalium* WGS (nucleotide accession no. CP120510.1; 34,273-bp spanning nucleotides from 26,385 to 60,657). The other structure similar with ICE*UB37* [27,974/28,781 (97.20%)] was also confirmed in *S. macedonicus* WGS (nucleotide accession no. CP113440.1; 28,775-bp spanning nucleotides from 1,544,153 to 1,572,927).

Another streptococcal ICE resembling *S. uberis* ICEs was *S. suis* ICE*nsui78*–*tet*(O)–*erm*(B) [33]. However, *S. suis* ICE*nsui78* seems incomplete, because of lacking VirB4, coupling protein VirD4, and replication initiator protein A in the ICE. This could be possibly resulting from shotgun WGS using Illumina that did not fully resolve the ICE. Additionally, we analyzed the conjugation family [34] to which ICE*UB37* belongs. This ICE fits into the Tn*5252* (conjugation family) (Supplementary Fig. 3).

In conclusion, for full ICE characterization in *S. uberis* with WGSs, a comparative genomic analysis is required with use of ICEfinder and other annotation tools.

Limitations

Characterizing ICEs of selected *S. uberis* strains ($n=22$) only by short-read WGS technology may be a limitation as repetitive sequences may be dismissed. Given the significant clinical implications of this research, it is recommended that whole-genome sequencing needs to be conducted using long-reads to enable more meaningful linear comparisons. We may consider requesting an extension for further modifications to solve the limitations of this study. Creations of hybrid genomes on two platforms making long- and short-read WGSs should be performed to completely identify ICEs. To the best of our knowledge, conjugation experiments using *S. uberis* have not been reported. We need to perform in vitro conjugation experiments to confirm ICE transfer between selected donor and recipient strains. Detailed information (antimicrobial dosing and duration) should be clarified for the relationships between ICEs and their clinical implications.

Abbreviations

AMR	Antimicrobial resistance
ATCC	American Type Culture Collection
Blastx	Basic Local Alignment Search Tool X
CAGECAT	CompArative GEne Cluster Analysis Toolbox
CDS	Coding DNA sequence
CLSI	Clinical and Laboratory Standards Institute
CRISPR	Clustered regularly interspaced short palindromic repeats
DFAST	DDBJ Fast Annotation and Submission Tool
ICE	Integrative and conjugative element
MGE	Mobile genetic element
NCBI	National Center for Biotechnology Information
PCR	Polymerase chain reaction
PGAP	Prokaryotic Genomes Annotation Pipeline
WGS	Whole-genome sequence

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13104-024-07065-3.

Supplementary Material 1

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Author contributions

Each author is expected to have made substantial contributions. The study was conceived and designed by TT, TM, and YT. The data were collected by TM and TT. The data were analyzed by TM, HY, MG, and YT. The manuscript was drafted by TT. The manuscript was critically revised by TT and TM. All authors read and approved the final manuscript.

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Data availability

Sequence data that support the findings of this study have been deposited in NCBI, US. Supplementary Table 2 lists the corresponding GenBank nucleotide accession numbers of WGSs.

Declarations

Ethics approval and consent to participate

The ethics committee of the Sanritsu Zelkova Veterinary Laboratory reviewed and approved our study design to maintain the anonymity and privacy of companion animals (approval no. SZ20220324). Background information (host species, collection year, geographic location, and isolation source) for the WGSs is available in the NCBI database. A total of twenty-two isolate-related background information was enrolled in the study. The consent to participate is not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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